

CLAIMS

1. The use of a mutagenic agent blocking the DNA replication in the cell for in vitro inserting a nucleic acid of interest within a predetermined nucleotide sequence present in a chromosome contained in a prokaryotic or eukaryotic cell, said nucleic acid of interest being, prior to its insertion, included in a DNA vector replicating in said prokaryotic or eukaryotic host cell.

2. The use according to claim 1, characterized in that the mutagenic agent is selected amongst N-acetoxy-2-acetylaminofluorene (N-AcO-AAF), an alkylating agent, benzo(a) pyrene-diol-epoxyde (BPDE) as well as a UV irradiation.

3. The use according to claim 2, characterized in that the mutagenic agent is N-acetoxy-2-acetylaminofluorene (N-AcO-AAF).

4. A method for in vitro inserting a nucleic acid of interest initially included in a DNA vector, within a predetermined nucleotide sequence present in a chromosome contained in a prokaryotic or eukaryotic cell, characterized in that it comprises the following steps of:

a) contacting the DNA vector comprising the nucleic acid of interest, and replicating in said prokaryotic or eukaryotic cell, with a mutagenic agent blocking the DNA replication in the cell;

b) transfecting prokaryotic or eukaryotic cells with the DNA vector such as obtained at the end of step a); and

c) selecting the prokaryotic or eukaryotic cells for which the nucleic acid of interest has been integrated into the predetermined nucleotide sequence.

5. A method according to claim 4, characterized in that it further comprises the following step of:

d) selecting, amongst the prokaryotic or eukaryotic cells as selected in step c), the cells wherein the DNA vector sequences, other than those of the nucleic acid of interest, were removed.

6. A method according to claim 4, characterized in that the mutagenic agent is selected amongst N-acetoxy-2-acetylaminofluorene (N-AcO-AAF), an alkylating agent, benzo(a) pyrene-diol-epoxyde (BPDE) as well as a UV irradiation.

7. A method according to claim 5, characterized in that the mutagenic agent is N-acetoxy-2-acetylaminofluorene (N-AcO-AAF).

8. A method according to claim 7, characterized in that in step a), the N-AcO-AAF is contacted with the DNA vector comprising the nucleic acid of interest, at a concentration adapted for binding at least 10 N-AcO-AAF molecules per molecule of the polynucleotide.

9. A method according to claim 8, characterized in that the concentration in N-AcO-AAF is adapted for binding at least 50 N-AcO-AAF molecules per molecule of the polynucleotide.

10. A method according to any one of claims 4 to 9, characterized in that the nucleic acid of interest to be inserted into the genome of the prokaryotic or eukaryotic cell, being initially included in said DNA vector comprises respectively at its end 5' and at its end 3', sequences with a high identity degree with the corresponding sequences located at the ends 5' and 3' of the target DNA contained in the chromosome.

11. A method according to claim 10, characterized in that the sequences respectively located at end 5' and at end 3' of the nucleic acid of interest are identical respectively to the ends 5' and 3' of the target DNA contained in the chromosome.

12. A method according to any one of claims 4 to 11, characterized in that the nucleic acid of interest included in said DNA vector comprises a selection marker nucleotide sequence.

13. A method according to any one of claims 4 to 12, characterized in that the nucleic acid of interest comprises an open reading frame coding a protein of therapeutic interest.

14. A method according to any one of claims 4 to 12, characterized in that the nucleic acid of interest comprises an open reading frame disrupted by a heterologous nucleotide sequence.

15. A method according to any one of claims 4 to 13, characterized in that the nucleic acid of interest codes an antisense RNA.

16. A method according to any one of claims 13 to 15, characterized in that the nucleic acid of interest further comprises a nucleotide sequence with a promoter function, being functional in the selected prokaryotic or eukaryotic host cell, under the control of which the

open reading frame or the sequence coding the RNA included in said nucleic acid of interest is arranged.

17. A method according to any one of claims 4 to 16, characterized in that the polynucleotide comprising the nucleic acid of interest comprises
5 a marker nucleotide sequence located, in said polynucleotide, outside the nucleotide sequence of the nucleic acid of interest.

18. A method according to any one of claims 4 to 17, characterized in that said DNA vector is a bacterial plasmid.

19. A method according to any one of claims 4 to 18, characterized
10 in that said DNA vector is a plasmid being functional in bacterial cells.

20. A method according to any one of claims 4 to 18, characterized in that said DNA vector is a plasmid being functional in human cells.

21. A method according to any one of claims 4 to 20, characterized in that the DNA vector is a double strand linear DNA.

15 22. A method according to any one of claims 4 to 21, characterized in that the cells transfected in step b) comprise bacterial cells.

23. A method according to any one of claims 4 to 21, characterized in that the cells transfected in step b) consist in non human mammalian cells.

20 24. A method according to any one of claims 4 to 21, characterized in that the cells transfected in step b) consist in human cells.